In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 4, lines 1-6, and replace it with the following paragraph:

In yet another aspect of the present invention, the first single chain Fv molecule and the immunoglobulin light chain constant region domain are covalently linked via a first peptide linker which preferably comprises the amino acid sequence EPKSADKTHTCPPCPGGGS (SEQ ID NO: 1), and the second single chain Fv and the immunoglobulin heavy chain constant region domain are covalently linked via a second peptide linker which preferably comprises the amino acid sequence EPKSCDKTHTCPPCPGGGS (SEQ ID NO: 2).

Please delete the paragraph on page 14, lines 1-18, and replace it with the following paragraph:

In another preferred embodiment, the first polypeptide of the multivalent target binding protein comprises a first scFv molecule covalently linked to an immunoglobulin light chain fragment which comprises the variable region VL and the constant region CL, and the second polypeptide of the multivalent target binding protein comprises a second scFv molecule covalently linked to an immunoglobulin heavy chain fragment which comprises the variable region VH and the constant region CH1. *See* Figure 2. The VL region and VH region associate to form a target binding site. The CL region and CH1 region associate with each other to stabilize the multivalent target binding protein. Preferably, the first scFv molecule and the CL region are covalently linked via a first peptide linker which preferably consists of about 4 to about 15 amino acid residues. The second scFv molecule and the CH1 region are also preferably covalently linked via a second peptide linker which preferably consists of about 4 to about 15 amino acid residues. Preferably, the first peptide linker may have the amino acid sequence GGGS (SEQ ID NO: 3) or EPKSADKTHTCPPCPGGGS (SEQ ID NO: 1), and the second peptide linker may have the amino acid sequence EPKSCGGGS (SEQ ID NO: 4) or EPKSCDKTHTCPPCPGGGS (SEQ ID NO: 2). More preferably, the cysteine residue in the

second peptide linker may form a disulfide bond with the CL region in a manner similar to the disulfide bond formed between an antibody light chain and heavy chain. The molecular weight of the multivalent target binding protein of this embodiment may be about 100 kDa.

B2 Cont'd

Please delete the paragraph on page 16, lines 12-16, and replace it with the following paragraph:

B3

The peptide linkers for the scFv molecules of the multivalent target binding protein preferably consist essentially of Gly and Ser residues. A preferred peptide linker is $[GGGGS]_3$ (SEQ ID NO: 5). Glu and Lys residues may also be included. Suitable peptide linkers for a scFv molecule may be designed in accordance with the methods disclosed in US Patent No. 4,946,778, which is hereby incorporated by reference.

Please delete the paragraph on page 17, lines 1-26, and replace it with the following paragraph:

B4

In another embodiment, the first polypeptide of the multivalent target binding protein comprises a first scFv molecule covalently linked via a first peptide linker to an immunoglobulin light chain fragment, and the second polypeptide of the multivalent target binding protein comprises a second scFv molecule covalently linked via a second peptide linker to an immunoglobulin heavy chain fragment. The first and second peptide linkers may increase the flexibility of the scFv molecules with respect to other parts of the multivalent binding protein. This flexibility becomes significant when one target binding event hinders another target binding event due to , for example, the large size of the target. In a preferred embodiment, the immunoglobulin light chain fragment comprises the VL and CL regions, and the immunoglobulin heavy chain fragment comprises the VH and CH1 regions. The first and second peptide linkers preferably comprise at least 4 amino acid residues, more preferably at least 10 amino acid residues, and most preferably at least 15 amino acid residues. Preferably, the second peptide linker comprises a cysteine residue which is capable of forming a disulfide bond with the Cys 214 (Kabat's numbering) of the CL region of the immunoglobulin light

chain fragment. For example, the second peptide linker may have the amino acid sequence EPKSCGGGS (SEQ ID NO: 4), and the first peptide linker may have the amino acid sequence GGGS (SEQ ID NO: 3). For another example, the second peptide linker may have the amino acid sequence EPKSCDKTHTCPPCPGGGS (SEQ ID NO: 2), and the first peptide linker may have the amino acid sequence EPKSADKTHTCPPCPGGGS (SEQ ID NO: 1).

Please delete page 37, and replace it with the following page 37:

The primer 734VLscFv5'(Cys) has the sequence of:

5' TCTCTGCAGAGCCCAAATCTTGTGGTGGCGGTTCACAGCTGGTTGTGACTCAG 3' (SEQ ID NO: 6)

P K S C G G G S Q L V V T Q (SEQ ID NO: 7)

It represents the sense-strand sequence encoding the first four residues (PKSC) (SEQ ID NO: 15) of the human IgG1 hinge, linked in-frame to the first six residues (QLVVTQ) (SEQ ID NO: 24) of 734 VL, via a short flexible linker, GGGS (SEQ ID NO: 3). One Cys of the human hinge was included because it is required for the interchain disulfide linkage between the hMN14 heavy chain Fd-734scFv fusion and the hMN14 light chain. A Pst1 site was incorporated (underline) to facilitate ligation at the intronic sequence connecting the CH1 domain and the hinge.

The primer 734VLscFv3' has the sequence of:

5' AGCCTCCGCCTCCTGATCCGCACCTCCTAAGATCTTCAGTTTGGTTCC 3' (SEQ ID NO: 8)

G G G G S G G G G L I K L K T G (SEQ ID NO: 9)

It represents the anti-sense sequence encoding the last six residues (TKLKIL) (SEQ ID NO: 10) of the 734 VL domain, and part of the flexible linker sequence (GGGGSGGGG) (SEQ ID NO: 25), which is fused in-frame downstream of the VL domain.

The PCR-amplified product (~400 bp) was first treated with T4 DNA polymerase to remove the extra A residue added to the termini during PCR-amplification, and was subsequently digested with Pst1. The resultant product was a double-stranded DNA fragment with a Pst1 overhang and a blunt end.

PCR amplification of 734VH was performed using the primer set 734VHscFv5' and 734VHscFv3'(Sac1).

The primer 734VHscFv5' has the sequence of:

5' CCGGAGGCGGTGGGAGTGAGGTGAAACTGCAGGAGT 3' (SEQ ID NO: 11)

S G G G G S E V K L Q E (SEQ ID NO: 12)

It represents the sense-strand sequence encoding the remaining part of the flexible linker sequence (SGGGGS) (SEQ ID NO: 26) connecting the VL and VH sequences, and the first six residues (EVKLQE) (SEQ ID NO: 27) of the 734 VH domain.

The primer 734VHscFv3'(Sac1) has the sequence of:

B5 Cont'd

5'AACCTTGAGCTCGGCCGTCGCACTCATGAGGAGACGGTGACCGT 3' (SEQ ID NO: 13)

• S S V T V T (SEQ ID NO: 14)

Please delete the paragraph on page 38, lines 1-4, and replace it with the following paragraph:

B6

It represents the anti-sense sequence encoding the last six residues (TVTVSS) (SEQ ID NO: 14) of 734 VH. Also included is a translation stop codon (*). At position downstream of the stop codon, the restriction sites Eag1 (bold) and Sac1(underlined) were incorporated to facilitate subcloning.

Please delete the paragraph on page 38, lines 17-30, thru page 39, lines 1-2, and replace it with the following paragraph:

B7

Since the genomic SacII fragment for IgG1 only included part of the 5' intron sequence flanking the CH1 domain, the full intronic sequence was restored by inserting the remaining intronic sequence as a BamH1/SacII segment, into the corresponding sites of the CH1-734pSK. The BamH1/Eag1 fragment containing the full 5' intron, CH1 domain, connecting intron, 5 hinge-residues, short GGGS (SEQ ID NO: 3) linker, and a 734scFv sequences was then isolated, and used to replace the HindIII/Eag1 segment containing the human genomic IgG1 constant sequence in the hMN14pdHL2 vector. The hMN14pdHL2 vector was described in Leung SO, Losman MJ, Qu Z, Goldenberg DM and Hansen HJ, Enhanced Production of a Humanized Anti-carcinoembryonic Antigen Antibody, *Tumor Targeting* 2:184(#95) (1996). For pdHL2 vector, please see Losman MJ, Qu Z, Krishnan IS, Wang J, Hansen HJ, Goldenberg DM and Leung SO, Generation and Monitoring of cell lines producing humanized antibodies, *Clin. Cancer Res.*, 5:3101s-3105s (1999), and Losman MJ, Hansen HJ, Dworak H, Krishnan IS, Qu

Z, Shih LB, Zeng L, Goldenberg DM and Leung SO, Generation of a high-producing clone of a humanized anti-B-cell lymphoma monoclonal antibody (hLL2), *Cancer* (suppl), 80:2660-2666 (1997). These references, as well as any cited references in this disclosure, are hereby incorporated by reference.

Please delete page 39 lines 3-31, and replace it with the following paragraph:

A HNB linker with a BamH1 overhang on one end and a HindIII overhang on the other was used to facilitate the BamH1/Eag1 fragment ligation into the HindIII/Eag1 site in the hMN14pdHL2 vector. It has the sequence of:

5' AGCTTGCGGCCGC 3' (SEQ ID NO: 16)

3' ACGCCGGCGCTAG 5' (SEQ ID NO: 17)

The resultant vector is designated as hMN14-734pdHL2.

To insert a 734 scFv to the C-terminal end of the kappa chain for hMN-14 Fab, a similar strategy is used and described as follows:

A Sac1 fragment containing part of the 5' intron flanking the human CK domain, and most of the CK region sequence was co-ligated into the Sac1/BamH1 cloning site of a pBlueScript vector in the presence of a linker, CKSB. The CKSB linker contains two synthetic DNA nucleotide, which, when annealed, will generate a double stranded DNA encoding the last 13 amino acid of the human CK region, fused in-framed to the first 4 residues of the human IgG1 hinge, at the C-terminal of which attached a short flexible linker (GGGS) (SEQ ID NO: 3). The CKSB linker has the double-stranded sequence of: (SEQ ID NOS 18-19, respectively in order of appearance)

5' CGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTGAGCCCAAATCTGGTGGCG 3'

3' TCGAGCGGGCAGTGTTTCTCGAAGTTGTCCCCTCTCACACTCGGGTTTAGACCACCGCCTAG 5'

S P V T K S F N R G E C E P K S G G G S

The Sac1 3' overhang of the CKSB linker will ligate to the C-terminal Sac1 of the CK fragment, while the BamH1 end will ligate to the corresponding BamH1 site of the pBlueScript vector. The resultant staging vector is designated as CK(B)pSK.

The VL region of 734 was PCR-amplified with the primer set 734VLscFv5'(BglII) and 734VLscFv3'. The primer 734VLscFv5' (BglII) has the sequence of:

5' TCTAGATCTCAGCTGGTTGTGACTCAG 3' (SEQ ID NO: 20)

S Q L V V T Q (SEQ ID NO: 21)

It represents the sense-strand sequence encoding the first six residues (QLVVTQ) (SEQ ID NO: 24) of 734 VL. A 5' BglII site was incorporated (underlined) to facilitate subsequent ligation to the short flexible linker connecting to the CK domain.

The sequence of the 734VLscFv3' has been previously described.

Please delete the paragraph on page 40, lines 6-9, and replace it with the following paragraph:

The sequence of 734VHscFv3'(Sal1) is basically the same as 734VHscFv3'(Sac1) except that the Sac1 site was replaced by Sal1 (underlined).

5' AACCCTTGTCGACGGCCGTCGCACTCATGAGGAGACGGTGACCGT 3' (SEQ ID NO: 22)

* S S V T V T (SEQ ID NO: 23)

In the Claims:

Please amend the claims as follows:

8. (Amended) The target binding protein of claim 7, wherein the first peptide linker comprises the amino acid sequence EPKSADKTHTCPPCPGGGS (SEQ ID NO: 1), and wherein the second peptide linker comprises the amino acid sequence EPKSCDKTHTCPPCPGGGS (SEQ ID NO: 2).

REMARKS

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. No new matter has been added.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.